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## PAPER

# The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects

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**OBJECTIVE:** To study the effects of 13 weeks conjugated linoleic acid (CLA) supplementation in overweight subjects after weight loss on weight regain, body composition, resting metabolic rate, substrate oxidation, and blood plasma parameters.

**DESIGN:** This study had a double-blind, placebo-controlled randomized design. Subjects were first submitted to a very-low-calorie diet (VLCD 2.1 MJ/d) for 3 weeks after which they started with the 13-week intervention period. They either received 1.8 g CLA or placebo per day (low dosage, LD) or 3.6 g CLA or placebo per day (high dosage, HD).

**SUBJECTS:** A total of 26 men and 28 women (age  $37.8 \pm 7.7$  y; body mass index (BMI)  $27.8 \pm 1.5$  kg/m<sup>2</sup>).

**MEASUREMENTS:** Before VLCD ( $t = -3$ ), after VLCD but before CLA or placebo intervention ( $t = 0$ ) and after 13-week CLA or placebo intervention ( $t = 13$ ), body weight, body composition (hydrodensitometry and deuterium dilution), resting metabolic rate, substrate oxidation, physical activity, and blood plasma parameters (glucose, insulin, triacylglycerol, free fatty acids, glycerol and  $\beta$ -hydroxy butyrate) were measured.

**RESULTS:** The VLCD significantly lowered body weight ( $6.9 \pm 1.7\%$ ), %body fat, fat mass, fat-free mass, resting metabolic rate, respiratory quotient and plasma glucose, insulin, and triacylglycerol concentrations, while free fatty acids, glycerol and  $\beta$ -hydroxy butyrate concentrations were increased. Multiple regression analysis showed that at the end of the 13-week intervention, CLA did not affect %body weight regain (CLA LD  $47.9 \pm 88.2\%$ , CLA HD  $27.4 \pm 29.8\%$ , Placebo LD  $32.0 \pm 42.8\%$ , Placebo HD  $22.5 \pm 37.9\%$ ). The regain of fat-free mass was increased by CLA (LD  $6.2 \pm 3.9$ , HD  $4.6 \pm 2.4\%$ ) compared to placebo (LD  $2.8 \pm 3.2\%$ , HD  $3.4 \pm 3.6\%$ ), independent of %body weight regain and physical activity. As a consequence of an increased regain of fat-free mass by CLA, resting metabolic rate was increased by CLA (LD  $12.0 \pm 11.4\%$ , HD  $13.7 \pm 14.4\%$ ) compared to placebo (LD  $9.1 \pm 11.0\%$ , HD  $8.6 \pm 8.5\%$ ). Substrate oxidation and blood plasma parameters were not affected by CLA.

**CONCLUSION:** In conclusion, the regain of fat-free mass was favorably, dose-independently affected by a 13-week consumption of 1.8 or 3.6 g CLA/day and consequently increased the resting metabolic rate. However, it did not result in improved body weight maintenance after weight loss.

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**Keywords:** conjugated linoleic acid; CLA; body weight maintenance; body composition; resting metabolic rate; humans

## Introduction

Conjugated linoleic acid (CLA) is naturally found in beef, milk and milk products since it is an intermediate in the

biohydrogenation of linoleic acid that occurs in the rumen by bacteria.<sup>1,2</sup> CLA refers to a group of positional and geometrical isomers of linoleic acid containing conjugated double bonds. The natural form is predominantly the *cis*-9, *trans*-11 isomer.

Numerous physiological effects in relation to body weight control have been attributed to CLA in animals. In different animal models, CLA has been shown to reduce body fat<sup>3–10</sup> and to increase lean body mass.<sup>3,5–7</sup> However, effects on body weight are controversial. Some investigators found

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reduced body weight after a CLA diet,<sup>4,5,7</sup> whereas others found no effect<sup>3,6,9–11</sup> or an increase in body weight.<sup>11</sup> Furthermore, CLA intake has been associated with an increased energy expenditure.<sup>4,9,12</sup> Only a few human studies have been conducted to study the effect of CLA on body weight, body mass index (BMI) and/or fat mass. Even though fat mass<sup>13,14</sup> and sagittal abdominal diameter<sup>15</sup> were lowered by CLA, it did not result in body weight loss.<sup>13–18</sup> These studies assessed weight loss or loss of fat mass. However, the effects of CLA might appear more clearly while subjects are in a state of weight (re)gain, since CLA reduces fat uptake into adipocytes by lowering the lipoprotein lipase activity<sup>3,5</sup> as well as stearoyl-CoA desaturase,<sup>19</sup> rather than enhancing lipolysis<sup>20</sup> and therefore it could block body fat gain instead of reducing body fat level. At the time of the beginning of the present study, the effect of CLA on substrate oxidation and resting metabolic rate was not investigated in humans. The aim of this study was to investigate the effect of two dosages of CLA after weight loss on body weight maintenance, substrate oxidation, and resting metabolic rate. We hypothesized that CLA might affect body composition, that is, reduce regain of body fat mass and enhance regain of fat-free mass, which in turn might affect resting metabolic rate and therefore improve weight maintenance.

## Material and methods

### Subjects

In all, 60 overweight men and women (BMI between 25 and 30 kg/m<sup>2</sup>) aged between 20 and 50 y were recruited by advertisements in local newspapers and participated in this study. Selection was based upon being healthy and at least 3 months weight stable prior to the study, no use of any medication known to affect body weight and/or appetite, being nonsmoking, and at most moderate alcohol users (max 10 glasses/week). Subjects had to be unrestrained eaters. The degree of dietary restraint was determined by the three-factor eating questionnaire (TFEQ, score Factor 1, ie cognitive restraint  $\leq 9$ )<sup>21,22</sup> and by the Herman/Polivy restraint questionnaire (HP, score  $\leq 15$ ).<sup>23</sup> Height was measured using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). Body weight (in underwear) was measured on a digital balance (Seca, model 707, Hamburg, Germany; weighing accuracy of 0.1 kg) in fasted state and after voiding their bladder. BMI was calculated as weight/(height<sup>2</sup>). A total of 54 subjects completed the study. Of these, 6 subjects dropped out for several reasons: one subject for illness not related to the treatment, one subject because of use of medication, and four subjects because of motivation reasons. In all, 27 subjects (15 women and 12 men) completed the low-dosage study (LD) and 27 (13 women and 14 men) subjects completed the high-dosage study (HD). Both studies were run concurrently.

All subjects gave their written informed consent. The study was approved by the Medical Ethics Committee of Maastricht University.

### Intervention protocol

The study had a randomized placebo-controlled and double-blind design.

Before the intervention period, all subjects were submitted to a 3-week very-low-calorie diet (VLCD, 2.1 MJ; Modifast, Novartis). After 3 weeks on a VLCD, subjects started the intervention period. In order to achieve equi-caloric supplementation subjects were randomized to study I (LD trial) or study II (HD trial). In study I, subjects were randomized to 1.8 g CLA (Tonalin™ CLA 75% TG, Tonalin™, Hovdebygd, Norway) (three capsules/day with 600 mg CLA/capsule,  $n = 14$ ) or 1.8 g placebo (oleic acid, three capsules/day with 600 mg oleic acid/capsule,  $n = 13$ ) to be taken before breakfast, lunch, and dinner. In study II, subjects were randomized to 3.6 g CLA (six capsules/day with 600 mg CLA/capsule,  $n = 13$ ) or 3.6 g placebo (oleic acid, six capsules/day with 600 mg oleic acid/capsule,  $n = 14$ ) to be taken before breakfast, lunch, and dinner. The duration of the intervention period in both studies was 13 weeks.

The setup of study I and study II was the same, so that in case of no-dosage effect, the CLA groups could be pooled as well as the placebo groups.

### Test protocol

Before the VLCD (week -3), after VLCD but before intervention (week 0), during (weeks 3 and 8) and at the end of the intervention period (week 13), subjects came after an overnight fast to the university. On each visit body weight was measured. A fasting blood sample was taken and body composition, resting metabolic rate, substrate oxidation as respiratory quotient, and physical activity were measured on week -3, 0, and 13.

Body weight (in underwear or swimming clothes) was measured on a digital balance (Seca, model 707, Hamburg, Germany; weighing accuracy of 0.1 kg). Subjects were in fasted state and voided their bladder before measuring. Height was measured to the nearest 0.001 m using a wall-mounted stadiometer (SECA, Hamburg); BMI (kg/m<sup>2</sup>) was calculated as body weight (kg) divided by height (m) squared. Body composition was determined by hydrodensitometry and deuterium dilution (<sup>2</sup>H<sub>2</sub>O) technique<sup>24</sup> and was calculated using the combined equation of Siri.<sup>25</sup> Whole body density was determined by underwater weighing with simultaneous assessment of long volume residual with the helium dilution technique (Volugraph 2000, Mijndhardt, the Netherlands). The dilution of the deuterium isotope is a measure for total body water.<sup>26</sup> Subjects were asked to collect a urine sample in the evening just before drinking a weighed amount of deuterium-enriched water solution. After ingestion of the deuterium solution no further fluid or food consumption was permitted. At 10 h after ingestion of the deuterium solution, a second urine sample (second voiding) was collected. Deuterium concentration in the urine samples was measured using an isotope ratio mass spectrometer (Micromass Optima, Manchester, UK). Total body water was obtained by dividing the measured deuterium dilution space

by 1.04.<sup>24</sup> Body composition was calculated from the three-component model by Siri.<sup>25</sup> A total of 10 subjects did not undergo underwater weighing, so body composition was determined only with deuterium dilution.

Resting metabolic rate and respiratory quotient were measured after an overnight fast for at least 30 min. Oxygen consumption and carbon dioxide production were measured using a computerized, open-circuit, ventilated hood system. Expired gases were analyzed using a paramagnetic oxygen analyzer (Servomex, Type 500A, Crowborough Sussex, UK) and an infrared carbon dioxide analyzer (Servomex, Type 12-X1). The system was similar to the analysis system for the respiration chambers described before.<sup>27</sup> Calculation of resting metabolic rate was based upon the equation of Weir.<sup>28</sup> Respiratory quotient was calculated as carbon dioxide production divided by oxygen consumption.

In order to distinguish a possible effect of CLA on fat-free mass from a possible increased physical activity effect, objective assessment of physical activity was obtained using the Computer Science and Applications Inc. (CSA) activity monitor (model 7164). CSA is a small and light ( $5 \times 4 \times 1.5$  cm<sup>3</sup>, 43 g) uniaxial accelerometer that is designed to detect normal body movements. Owing to limiting problems, only subjects of the HD-group were asked to wear the CSA for 1 week at the beginning of the VLCD (week -3), during the first week of intervention (week 1) and during the last week of intervention (week 13). The monitor was held in place by an elastic belt at the lower back, that is, as close as possible to the center of gravity. Subjects were instructed to put on the CSA monitor as quickly as possible after waking up and to put it off before going to bed. Also, it could not be worn during water activities. The same monitor was used for each subject on each test occasion, and after each testing session the activity monitor was immediately removed and data downloaded.

The blood samples were mixed with EDTA to prevent clotting. Plasma was obtained by centrifugation (4°C, 3000 rpm, 10 min) and stored at -80°C until analysis of glucose by a hexokinase method (Roche Diagnostics, Hoffman-La Roche, Basel, Switzerland), triglycerides by the method of McGowan (GPO-trinder 337, Sigma, Moudebygda), glycerol by a glycerolkinase-lipase method (Boehringer, Mannheim, Germany), free fatty acids by an ACS-ACOD method (Wako chemicals, Neuss, Germany),  $\beta$ -hydroxy butyrate by the method of Moore *et al.*<sup>29</sup> using a semiautomated centrifugal spectrophotometer (Cobas Fara, Roche Diagnostics, Moudebygda), and insulin with ELISA (Merco-dia 10-1113-01).

### Statistics

Possible differences between subjects of the low and high CLA and placebo intervention groups for baseline characteristics (age, body weight, BMI, %body fat, and dietary restraint) were analyzed with a factorial ANOVA (Statview SE Graphics™, Moudebygda).

Changes in body weight, BMI, %body fat, fat mass, fat-free mass, resting metabolic rate, respiratory quotient, physical activity (counts), and blood parameters from weeks -3 to 0 were tested with repeated measures of ANOVA (Statview SE Graphics™) for all groups together.

The effect of CLA at week 13 on the dependent variable %body weight regain was analyzed by linear multiple regression model with treatment (0=placebo; 1=CLA), gender (0=men; 1=women) and dosage (0=LD, 1=HD) as independent variables. Similarly, the effect of CLA at week 13 for the dependent variables plasma glucose, insulin, free fatty acids, glycerol, and  $\beta$ -hydroxy butyrate were analyzed by linear multiple regression model with treatment (0=placebo; 1=CLA), gender (0=men; 1=women) and dosage (0=LD, 1=HD), and the values of weeks -3 and 0 of those parameters as independent variables (SPSS Inc., Chicago, IL, USA). The effect of CLA at week 13 for the dependent variables fat mass, fat-free mass, resting metabolic rate, and respiratory quotient were analyzed by linear multiple regression model with treatment (0=placebo; 1=CLA), gender (0=men; 1=women), and dosage (0=LD, 1=HD), and the values of weeks -3 and 0 of those parameters as well as percentage of body weight regain as independent variables (SPSS Inc., Chicago, IL, USA). Finally, the effect of CLA on physical activity (counts) was analyzed by linear multiple regression model with treatment (0=placebo; 1=CLA), gender (0=men; 1=women) and the values of week -3 and 0 of those parameters as well as percentage of body weight regain as independent variables (SPSS Inc., Chicago, IL, USA). The regression coefficient (RC) with 95% confidence interval (CI) of the CLA intervention was calculated for each dependent variable.

The level of significance is set at  $P < 0.05$ . Data are presented as means and standard deviations.

### Results

At the start of the study, subjects of the four subgroups did not differ with respect to age, body weight, BMI, %body fat, and dietary restraint (Table 1).

As a consequence of the VLCD, body weight at week 0 was significantly lower compared to week -3 ( $P < 0.0001$ ). The mean weight loss was  $6.9 \pm 1.7\%$  of the original body weight. Also the BMI was decreased as a consequence of the VLCD ( $P < 0.0001$ ). Percentage of body fat ( $P < 0.0001$ ), as well as fat mass ( $P < 0.0001$ ) and fat-free mass ( $P < 0.0001$ ) were significantly decreased after VLCD compared to before. The VLCD did not affect physical activity ( $65.1 \pm 18.1$  vs  $67.6 \pm 22.3$  counts  $\times 10^3$ , NS) (Table 1). Furthermore, resting metabolic rate ( $P < 0.0001$ ) and the respiratory quotient ( $P < 0.0001$ ) were significantly lowered after the VLCD compared to before (Table 2). Plasma concentrations of glucose ( $P < 0.001$ ), insulin ( $P < 0.001$ ), and triglycerides ( $P < 0.0001$ ) were lowered after the VLCD. The plasma concentrations of glycerol ( $P < 0.05$ ), free fatty acids

**Table 1** Mean age and degree of dietary restraint before weight loss, and body weight, body mass index (BMI), body fat, fat mass, and fat-free mass before and after weight loss with a 3-week very-low-calorie diet (VLCD) and after 13 weeks intervention with CLA (1.8 or 3.6 g) or placebo (Plac 1.8 or 3.6 g oleic acid)

	1.8 g CLA (n = 14)	1.8 g Plac (n = 13)	3.6 g CLA (n = 13)	3.6 g Plac (n = 14)	RC <sup>a</sup>	CI <sup>a</sup>	P
Age (y)	40.9 ± 5.0	39.5 ± 7.7	36.2 ± 7.6	34.0 ± 9.1			
Dietary restraint (F1 <sup>b</sup> )	4.8 ± 2.9	5.4 ± 2.1	5.4 ± 2.9	4.3 ± 2.0			
Body weight (kg) week -3	83.5 ± 7.4	83.5 ± 8.9	86.8 ± 8.4	81.9 ± 9.3			
Body weight (kg) week 0	77.6 ± 6.4*	78.0 ± 8.1*	80.5 ± 7.7*	76.2 ± 8.3*			
Body weight (kg) week 13	81.0 ± 8.1	79.4 ± 8.5	82.4 ± 8.3	77.5 ± 9.1	13.9	-16.1-44.0	NS
BMI (kg/m <sup>2</sup> ) week -3	27.6 ± 1.1	28.0 ± 1.6	28.3 ± 1.7	27.6 ± 1.5			
BMI (kg/m <sup>2</sup> ) week 0	25.6 ± 1.1*	26.1 ± 1.4*	26.2 ± 1.7*	25.7 ± 1.4*			
BMI (kg/m <sup>2</sup> ) week 13	26.8 ± 1.2	26.7 ± 1.6	26.8 ± 1.7	26.3 ± 1.6	0.3	0.1-1.2	NS
Body fat (%) week -3	32.2 ± 7.5	33.2 ± 7.5	30.3 ± 7.6	32.8 ± 6.3			
Body fat (%) week 0	30.8 ± 6.9*	31.3 ± 8.1*	28.7 ± 7.7*	31.1 ± 7.1*			
Body fat (%) week 13	29.7 ± 5.9	30.8 ± 7.8	27.3 ± 7.4	30.1 ± 6.6	-0.8	-1.0 to 0.4	<0.05
Fat mass (kg) week -3	26.8 ± 6.1	27.4 ± 5.7	26.2 ± 6.3	26.6 ± 4.6			
Fat mass (kg) week 0	23.8 ± 5.3*	24.2 ± 5.5*	23.1 ± 6.4*	23.5 ± 5.3*			
Fat mass (kg) week 13	24.0 ± 4.5	24.2 ± 5.4	22.4 ± 6.0	23.2 ± 5.3	-0.4	-1.1 to 0.3	NS
Fat-free mass (kg) week -3	56.7 ± 8.9	56.1 ± 10.0	60.6 ± 9.8	55.3 ± 9.8			
Fat-free mass (kg) week 0	53.8 ± 8.1*	53.9 ± 10.0*	57.4 ± 8.7*	52.6 ± 9.1*			
Fat-free mass (kg) week 13	57.1 ± 7.7	55.3 ± 10.0	60.1 ± 9.3	54.4 ± 9.3	0.9	0.1-1.6	<0.05
PA (counts × 10 <sup>3</sup> ) week -3 <sup>c</sup>			69.5 ± 22.0	61.5 ± 14.3			
PA (counts × 10 <sup>3</sup> ) week 0 <sup>c</sup>			67.5 ± 22.9	67.7 ± 23.0			
PA (counts × 10 <sup>3</sup> ) week 13 <sup>c</sup>			77.6 ± 31.4	66.1 ± 23.3	8.6	-6.5-23.7	NS

<sup>a</sup>Regression coefficient (RC) and confidence interval (CI) for the CLA effect (1.8 and 3.6 g CLA/day, pooled groups) compared to placebo (1.8 and 3.6 g oleic acid/day, pooled groups), corrected for dosage (except for physical activity), gender, and %body weight regain.

<sup>b</sup>Measured with Factor 1 of the Three-Factor Eating Questionnaire.<sup>21</sup>

<sup>c</sup>Physical activity.

\*Significantly different compared to before the VLCD measured for all groups together ( $P < 0.0001$ ).

( $P < 0.01$ ), and  $\beta$ -hydroxy butyrate ( $P < 0.0001$ ) were increased by the VLCD (Table 3).

After 13 weeks intervention, the subjects of the CLA group had a body weight regain of  $40.2 \pm 69.3\%$  (LD  $47.9 \pm 88.2\%$ , HD  $27.4 \pm 29.8\%$ ) while the placebo group had a body weight regain of  $24.8 \pm 33.6\%$  (LD  $32.0 \pm 42.9\%$ , HD  $22.5 \pm 37.9\%$ ) (NS) (Table 1). Thus, body weight regain during the intervention (week 13) was not influenced by CLA. Moreover, both independent variables dosage (RC -11.5; CI -41.5 to 18.6, NS) and gender (RC -14.4; CI -44.5 to 15.7, NS) did not affect body weight regain.

After 13 weeks intervention, CLA (LD  $-3.0 \pm 6.6\%$ , HD  $-5.1 \pm 5.8\%$ ) compared to placebo (LD  $-1.6 \pm 4.6\%$ , HD  $-3.1 \pm 4.5\%$ ) significantly reduced %body fat ( $P < 0.05$ ), independent of %body weight regain (RC 0.0; CI 0.0-0.0, NS), dosage (RC -0.3; CI -1.0 to 0.4, NS), and gender (RC 0.7; CI -0.4 to 1.8, NS) (Table 1). Expressing body composition as absolute fat mass and fat-free mass, CLA (LD  $1.5 \pm 10.8\%$ , HD  $-2.8 \pm 7.6\%$ ) compared to placebo (LD  $0.2 \pm 5.3\%$ , HD  $-1.5 \pm 4.5\%$ ) appeared not to affect fat mass, nor did dosage (RC -0.5; CI -1.2 to 0.2, NS) or gender (RC 0.2; CI -0.7 to 1.0, NS), but significantly increased fat-free mass (LD  $6.2 \pm 3.9\%$ , HD  $4.6 \pm 2.4\%$ ) compared to placebo (LD

$2.8 \pm 3.2\%$ , HD  $3.4 \pm 3.6\%$ ) ( $P < 0.05$ ), independent of %body weight regain (RC -0.0; CI -0.0 to 0.0, NS), dosage (RC 0.3; CI -0.4 to 1.1, NS), and gender (RC -1.0; CI -2.7 to 0.7, NS) (Table 1). Furthermore, CLA (LD  $12.0 \pm 11.4\%$ , HD  $13.7 \pm 14.4\%$ ) increased resting metabolic rate compared to placebo (LD  $9.1 \pm 11.0\%$ , HD  $8.6 \pm 8.5\%$ ) ( $P < 0.05$ ), independent of %body weight regain (RC 0.0; CI -0.0 to 0.0, NS), dosage (RC 0.6; CI -0.1 to 1.4, NS), and gender (RC -0.9; CI -2.1 to 0.2, NS) (Table 2). However, the resting metabolic rate was not affected by CLA independent of fat-free mass (RC 0.6; CI -0.2 to 1.3, NS). Thus, resting metabolic rate was increased as a function of an increased fat-free mass (Figure 1). The respiratory quotient was not significantly affected by CLA compared to placebo ( $P < 0.1$ ; Table 2).

At the end of the intervention, the physical activity was not affected by CLA (Table 1; RC 8.6; CI -6.5 to 23.7, NS) or gender (RC -3.5; CI -18.0 to 11.01, NS), indicating that the increased resting metabolic rate as a function of fat-free mass was not because of a possible physical activity effect.

A 13-week intervention with CLA did not significantly affect plasma glucose, insulin, triglycerides, glycerol ( $P < 0.1$ ), free fatty acids ( $P < 0.1$ ), and  $\beta$ -hydroxy butyrate concentrations (Table 3).

**Table 2** Mean resting metabolic rate (RMR) and respiratory quotient (RQ) before and after weight loss with a 3-week very-low-calorie diet (VLCD) and after 13 weeks intervention with CLA (1.8 or 3.6 g) or placebo (Plac 1.8 or 3.6 g oleic acid)

	1.8 g CLA (n = 14)	1.8 g Plac (n = 13)	3.6 g CLA (n = 13)	3.6 g Plac (n = 14)	RC <sup>a</sup>	CI <sup>a</sup>	P
RMR (MJ) week -3	7.3 ± 0.6	7.2 ± 0.9	7.6 ± 0.9	7.4 ± 0.8	0.8	0.0–1.5	<0.05
RMR (MJ) week 0	6.7 ± 0.7*	6.8 ± 0.6*	6.9 ± 0.6*	6.8 ± 0.9*			
RMR (MJ) week 13	7.5 ± 0.9	7.4 ± 0.8	7.8 ± 1.2	7.4 ± 0.8			
RQ week -3	0.82 ± 0.04	0.83 ± 0.04	0.81 ± 0.06	0.82 ± 0.04	0.02	-0.00 to 0.05	NS
RQ week 0	0.77 ± 0.05*	0.78 ± 0.04*	0.78 ± 0.04*	0.76 ± 0.04*			
RQ week 13	0.84 ± 0.04	0.81 ± 0.05	0.87 ± 0.07	0.85 ± 0.03			

<sup>a</sup>Regression coefficient (RC) and confidence interval (CI) for the CLA effect (1.8 and 3.6 g CLA/day, pooled groups) compared to placebo (1.8 and 3.6 g oleic acid/day, pooled groups), corrected for dosage, gender, %body weight regain.

\*Significantly different compared to that measured before the VLCD for all groups together ( $P < 0.0001$ ).

**Table 3** Mean plasma glucose, insulin, triacylglycerol (TG), free fatty acids (FFA), glycerol, and  $\beta$ -hydroxy butyrate (BHB) concentrations before and after weight loss with a 3-week very-low-calorie diet (VLCD) and after 13 weeks intervention with CLA (1.8 or 3.6 g) or placebo (Plac 1.8 or 3.6 g oleic acid)

	1.8 g CLA (n = 14)	1.8 g Plac (n = 13)	3.6 g CLA (n = 13)	3.6 g Plac (n = 14)	RC <sup>a</sup>	CI <sup>a</sup>	P
Glucose (mmol/l) week -3	5.0 ± 0.4	5.0 ± 0.6	5.3 ± 0.6	5.3 ± 0.5	0.05	-0.09 to 0.19	NS
Glucose (mmol/l) week 0	4.6 ± 0.3*	4.7 ± 0.5*	5.0 ± 0.4*	4.9 ± 0.3*			
Glucose (mmol/l) week 13	5.0 ± 0.3	5.0 ± 0.4	5.2 ± 0.6	5.1 ± 0.4			
Insulin ( $\mu$ U/l) week -3	4.8 ± 2.5	3.1 ± 1.7	9.0 ± 2.2	7.6 ± 2.9	0.1	-1.4 to 1.7	NS
Insulin ( $\mu$ U/l) week 0	2.3 ± 1.9*	2.0 ± 1.2*	4.7 ± 1.6*	5.7 ± 2.5*			
Insulin ( $\mu$ U/l) week 13	4.1 ± 2.5	3.0 ± 1.7	7.9 ± 4.3	7.8 ± 4.4			
TG ( $\mu$ mol/l) week -3	1555 ± 864	1216 ± 1007	1192 ± 379	1123 ± 398	176	-38 to 390	NS
TG ( $\mu$ mol/l) week 0	828 ± 195*	757 ± 184*	753 ± 237*	809 ± 223*			
TG ( $\mu$ mol/l) week 13	1334 ± 704	1035 ± 564	1213 ± 584	1088 ± 447			
FFA ( $\mu$ mol/l) week -3	292 ± 122	325 ± 150	318 ± 168	306 ± 151	-74	-149 to 1	NS
FFA ( $\mu$ mol/l) week 0	372 ± 221*	440 ± 204*	321 ± 101*	429 ± 226*			
FFA ( $\mu$ mol/l) week 13	226 ± 92	298 ± 120	250 ± 104	307 ± 156			
Glycerol ( $\mu$ mol/l) week -3	73 ± 28	86 ± 34	93 ± 51	91 ± 48	-17	-35 to 1	NS
Glycerol ( $\mu$ mol/l) week 0	104 ± 66*	128 ± 76*	74 ± 25*	108 ± 70*			
Glycerol ( $\mu$ mol/l) week 13	70 ± 28	81 ± 24	52 ± 21	72 ± 40			
BHB ( $\mu$ mol/l) week -3	275 ± 72	239 ± 87	262 ± 55	258 ± 81	-22	-62 to 19	NS
BHB ( $\mu$ mol/l) week 0	852 ± 427*	687 ± 547*	496 ± 217*	646 ± 428*			
BHB ( $\mu$ mol/l) week 13	211 ± 51	218 ± 88	249 ± 73	250 ± 87			

<sup>a</sup>Regression coefficient (RC) and confidence interval (CI) for the CLA effect (1.8 and 3.6 g CLA/day, pooled groups) compared to placebo (1.8 and 3.6 g oleic acid/day, pooled groups), corrected for dosage, gender, and %body weight regain.

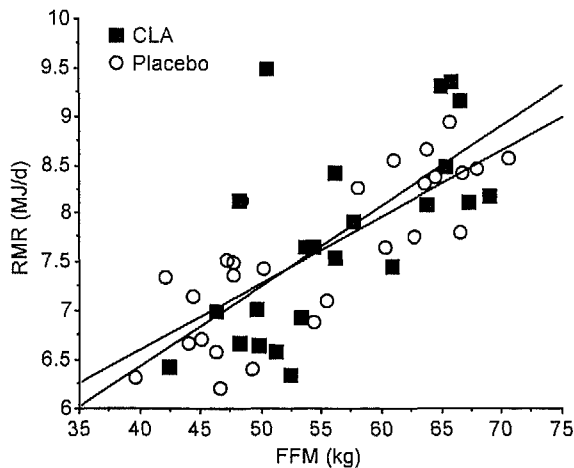
\*Significantly different compared to that measured before the VLCD for all groups together ( $P < 0.0001$ ).

## Discussion

The present study investigated the effect of CLA compared to placebo (oleic acid) after a 3-week VLCD (2.1 MJ/day) on body weight regain, parameters of body composition, resting metabolic rate, and respiratory quotient while physical activity was monitored. It was shown that a 13-week CLA supplementation after body weight loss lowered regain of body fat expressed as %body fat compared to placebo independent of % body weight regain. However, it showed that CLA affected regain of fat-free mass rather than regain of fat mass. As a result of its effect on fat-free mass, CLA

increased resting metabolic rate independent of a possible change in physical activity, since this was not significantly different from placebo. In contrast, CLA did not affect body weight regain, respiratory quotient, or blood parameters.

In different animal models, CLA has been shown to alter body composition, that is decrease fat mass<sup>3–10</sup> and increase fat-free mass,<sup>3,5–7</sup> while the effects in humans are inconsequent. Differences between humans and animals for the effect of CLA on body composition might be because of several factors, for example, dosage or length of intervention, although a recent publication suggests that differences



**Figure 1** Resting metabolic rate (RMR, MJ/day) as a function of fat-free mass (FFM, kg) after 13 weeks intervention with conjugated linoleic acid (CLA,  $n=23$ ;  $r^2=0.4$ ,  $P<0.001$ ) and placebo (oleic acid,  $n=27$ ;  $r^2=0.6$ ,  $P<0.0001$ ). Multiple regression analysis showed that the resting metabolic rate at week 13 was not affected by CLA independent of the fat-free mass (RC 0.6; CI  $-0.2$  to  $1.3$ , NS).

in metabolic rate might be of more importance than other factors.<sup>30</sup>

Even though in the present study, body weight regain was not affected by CLA, it was mainly because of an increase in fat-free mass and hardly of fat mass, which was even stronger in the groups using CLA. So, body composition rather than body weight was affected by CLA. This is in line with previous studies conducted in humans,<sup>13–15</sup> although the effects of CLA on body composition are inconsistent. Blankson *et al*<sup>13</sup> observed no effect from 12 weeks CLA supplementation (1.7, 3.4, 5.1 or 6.8 g/day) on body weight in overweight and obese subjects. Fat mass (kg), measured with DEXA, decreased with 1.7 and 5.1 g CLA/day, but not with the other concentrations. Furthermore, there was a trend in favor of increased fat-free mass in all groups receiving CLA, but reached only significance in the group receiving the highest dose. CLA had no effect on body weight or composition in a study of Berven *et al*.<sup>16</sup> They observed that after a daily consumption of 3.4 g CLA for 12 weeks there is no effect on body weight, BMI, or fat mass, measured with bio-impedance measures compared to placebo in obese subjects. Also, Zambell *et al*<sup>17</sup> found no effect of CLA (3 g/day, 9 weeks) on body weight and composition, measured by total body electrical conductivity. In contrast, a daily intake of 0.7 g CLA for 4 weeks followed by a 4-week 1.4 g CLA supplementation, lowered fat mass measured with skin fold thickness during the second period, but had no effect on the overall decrease in fat mass in a study of Mougios *et al*.<sup>14</sup> Furthermore, CLA did not influence body weight during the overall study, nor during the two periods. Body weight as well as sagittal abdominal diameter was not affected by CLA after a 12-week supplementation with 4.2 g/

day in a study by Smedman and Vessby.<sup>18</sup> However, a reduction of body fat, measured with skin fold thickness and bio-impedance, in the CLA-supplemented group was observed. Also, Riserus *et al*<sup>15</sup> studied the effect of 4.2 g CLA/day for 4 weeks on body fat distribution. Even though body weight and waist-hip ratio were not affected by CLA, in this study the sagittal abdominal diameter in obese men was lowered. However, more precise measures of fat mass and fat-free mass were not conducted. The inconsistent and contradictory results between the present and previous studies could be because of a number of factors, including differences in methodology and subject groups, the quantity and duration of CLA intake, the fatty acid composition of CLA, and also by the executed measurements for body composition and body fat distribution. In the present study, fat-free mass rather than fat mass affected %body fat, while in previous studies fat mass was often significantly reduced.<sup>13,14</sup> Since body weight was not reduced, but fat mass was, it is possible that previously CLA also (slightly) affected fat-free mass, although it might not have been sufficient to reach significance. Furthermore, in the present study, body composition was obtained by the three-compartment model while in previous mentioned studies accurate measurements for body fat as well as body fat distribution are missing.<sup>31</sup>

It was suggested that CLA affected body composition by lowering fat mass. It is known from *in vitro* studies that CLA causes a reduction in lipid uptake by adipose cells because of an effect on lipoprotein lipase<sup>3,5</sup> and stearoyl-CoA desaturase.<sup>19,20</sup> Moreover, the carnitine palmitoyltransferase activity in muscle cells, which is the rate-limiting enzyme in  $\beta$ -oxidation, is increased by CLA.<sup>3</sup> In other words, CLA lowers the uptake of lipids by adipocytes and stimulates fat oxidation in muscle cells. However, in the present study fat-free mass was mainly affected. Furthermore, overall fat oxidation, as obtained by RQ, was not decreased, but tended to increase. In animals,<sup>3</sup> as well as in humans,<sup>13</sup> the increase of lean body mass has been described previously, although the responsible mechanism is poorly understood. It has been suggested that the anabolic effect of CLA might be caused by changes in the regulation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and Interleukin-1 (IL-1).<sup>20</sup> In the present study, the increase in fat-free mass is not because of differences between the CLA and placebo groups in physical activity or energy intake, although CLA decreased appetite (Kamphuis *et al*, European Journal of Clinical Nutrition in press).

We found that CLA increased resting metabolic rate indirectly as a function of an increased fat-free mass, independent of dose, gender, and weight %regain. This finding is in contrast with a study by Zambell in which no effect of CLA on resting metabolic rate was observed in humans.<sup>17</sup> However, in the study by Zambell, CLA did not affect body composition. In mice, however, CLA was observed to increase energy expenditure.<sup>4,9,12</sup> Also here, the discrepancy between animal and human studies might be caused by differences in metabolic rate.<sup>30</sup>

There is growing evidence that the different isomers of CLA (c9,t11 and t10,c12) might have different effects.<sup>5,19,20,32</sup> The c9,t11 isomer is the principal dietary form of CLA (80–90%) and is incorporated into membranes, but the t10,c12 isomer seems to be the most important in energy metabolism.<sup>20</sup> In our study, a mixture of equal amounts of both isomers was used, so the effects of this study could result from either or both isomers.

In the present study, no difference between the LD and HD CLA for body composition and resting metabolic rate was observed. Compared to other studies, the dosages used in this study were rather low, but despite the low concentrations effects were observed. This is probably due to the circumstances during the CLA intervention. Since subjects are in a state of weight regain, the body might be more vulnerable for CLA compared to a state of weight stability. Apparently, a relatively LD of CLA, which is much higher than usual CLA intake (0.2 g/day<sup>33</sup>), affects fat-free mass already during weight regain, while a larger dosage hardly increases this effect.

In conclusion, 13 weeks supplementation with 1.8 or 3.6 g CLA/day after a 3-week VLCD was not effective in improving body weight maintenance after weight loss compared to placebo (1.8 or 3.6 g oleic acid/d), but affected dose independently body composition. CLA lowered regain of %body fat by increasing regain of fat-free mass, and consequently increased the resting metabolic rate.

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